K-*ras* Mutation, p53 Overexpression, and Microsatellite Instability in Biliary Tract Cancers: A Population-based Study in China

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ABSTRACT

Purpose: **The genetic alterations in biliary tract cancer and clinicopathological associations have not been studied in large population-based studies.**

Experimental Design: **We evaluated genetic alterations such as K-*ras* mutation, p53 overexpression, microsatellite instability (MSI), and alterations of the polyadenine tract present in the *transforming growth factor* β *receptor type II (TGF*β*RII)* gene in 126 biliary tract cancers: 75 gallbladder cancers, 33 bile duct cancers, and 18 ampullary cancers. These genetic alterations were compared with patient demographics and clinicopathological characteristics of the tumors.**

Results: **Mutation of the K-*ras* gene was present in 18 of 126 (14.3%) biliary tract cancers. K-*ras* mutation was present in 11 of 18 (61.1%) ampullary cancers, 5 of 33 (15.2%) bile duct cancers, and 2 of 75 (2.7%) gallbladder cancers ($P = 0.000001$). The mean survival of patients who had bile duct carcinomas with K-*ras* mutation was 3.0 ± 2.2 months compared with 15.5 ± 12.5 months for those without mutation ($P = 0.03$) but was not different for other tumor sites. p53 overexpression was present in 34 of 123 (27.6%) cancers. MSI-high (allelic shifts in 40% or more loci or alteration of the *TGF*β*RII* gene) was present in 4 of 126 (3.2%) biliary tract cancers without hereditary nonpolyposis colorectal cancer. MSI-high was more common in mucinous adenocarcinomas ($P = 0.006$) and in patients with early age of onset of cancer ($P = 0.04$).**

Conclusions: **The genetic alterations in biliary tract cancers are dependent on the tumor subsite, histology, and age of onset and are associated with prognosis.**

INTRODUCTION

Biliary tract carcinomas are relatively uncommon in most parts of the world, although an increased incidence is present in certain geographical locations and certain areas (1). From 1972 to 1994, biliary tract cancer had the most rapid rise in incidence of any malignancy in Shanghai, the People's Republic of China, with a 119% increase in men and a 124% increase in women (2). Reasons for the rising incidence of biliary tract cancers in Shanghai are unclear, although improvements in diagnosis and classification, as well as recent dietary and socioeconomic changes, may contribute to this trend. This rising incidence was present for all three sites of biliary tract carcinomas: gallbladder, extrahepatic bile duct, and ampulla of Vater.

The genetic alterations commonly observed in sporadic biliary tract cancers include mutations of the K-*ras* (3–13), *p16* (14), *APC* (13, 15), β-*catenin* (16), and *p53* (6, 15, 17, 18) oncogenes and tumor suppressor genes, p53 overexpression (19–22), and loss of heterozygosity of chromosomal arms (8, 23–25).

Molecular studies have identified cancers in which a primary genetic abnormality is defective DNA nucleotide mismatch repair (26–28). This abnormality results in extensive instability in repeated nucleotide sequences called microsatellites, a condition that is termed MSI[2] (also termed DNA replication error or ubiquitous somatic mutation; Refs. 27, 29, 30). MSI is caused by inactivation of one of a group of genes responsible for nucleotide mismatch repair, including *hMSH2*, *hMLH1*, *PMS1*, *PMS2*, *hMSH6/GTBP*, and *hMSH3* (27, 29–34). HNPCC is usually attributable to a germ-line mutation in *hMSH2* or *hMLH1* (30–34). Alterations of the 10-bp polyadenine repeats within the *TGF*β*RII* gene are present in the vast majority of MSI-H colorectal cancers (35, 36). MSI of dinucleotide repeats in biliary tract cancers has been described (37–39), but alterations of *TGF*β*RII* have not been reported (37, 38).

Although these genetic events have been evaluated extensively in biliary tract cancers from hospital-based studies, these events have not been studied in large, prospectively defined, population-based studies. Therefore, we studied the K-*ras* mutation, p53 overexpression, and MSI, including alteration of the *TGF*β*RII* gene, in biliary tract cancers from a population-based

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[2] The abbreviations used are: MSI, microsatellite instability; HNPCC, hereditary nonpolyposis colorectal cancer; TGFβRII, transforming growth factor β receptor type II.

Table 1 Prevalence of K-*ras* mutations compared with patient demographics and carcinoma characteristics in 126 biliary tract carcinomas

	K-*ras* mutation no. (%)							
	Absent				Present			
	Gallbladder (n = 73)[a]	Bile duct (n = 28)[a]	Ampulla (n = 7)[a]	All sites (n = 108)	Gallbladder (n = 2)[a]	Bile duct (n = 5)[a]	Ampulla (n = 11)[a]	All sites (n = 18)
Age (mean ± SD) yr	64.9 ± 9.3	64.0 ± 9.5	70.7 ± 3.4	64.9 ± 9.2	57.0 ± 22.6	57.4 ± 8.1	65.6 ± 6.3	62.4 ± 9.3
Gender								
Female	55 (75.3)	9 (32.1)	3 (42.9)	67 (62.0)[b]	1 (50.0)	2 (40.0)	3 (27.3)	6 (33.3)[b]
Male	18 (24.7)	19 (67.8)	4 (57.1)	41 (38.0)[b]	1 (50.0)	3 (60.0)	8 (72.7)	12 (66.7)[b]
Cholelithiasis								
Present	51 (69.9)	11 (39.3)	3 (42.9)	65 (60.2)[c]	1 (50.0)	1 (20.0)	2 (18.2)	4 (22.2)[c]
Absent	20 (27.4)	17 (60.7)	4 (57.1)	41 (38.0)[c]	1 (50.0)	3 (60.0)	9 (81.8)	13 (72.2)[c]
Unknown	2 (2.7)	0	0	2 (1.8)	0	1 (20.0)	0	1 (5.6)
Histological type, Adenocarcinoma								
Not otherwise specified	47 (64.4)	21 (75.0)	7 (100.0)	75 (69.4)	1 (50.0)	5 (100.0)	8 (72.7)	14 (77.7)
Papillary	10 (13.7)	1 (3.6)	0	11 (10.2)	1 (50.0)	0	1 (9.1)	2 (11.1)
Other adenocarcinomas	5 (6.8)[d]	2 (7.1)[d]	0	7 (6.5)[d]	0	0	1 (9.1)[d]	1 (5.6)[d]
Other histological types	11 (15.1)[e]	4 (14.3)[e]	0	15 (13.9)[e]	0	0	1 (9.1)[e]	1 (5.6)[e]
TNM[f] stage								
Stage I	14 (19.2)	0	0	14 (13.0)	0	1 (20.0)	0	1 (5.6)
Stage II	16 (21.9)	8 (28.6)	5 (71.4)	29 (26.9)	1 (50.0)	2 (40.0)	8 (72.7)	11 (61.1)
Stage III	14 (19.2)	5 (17.9)	2 (28.6)	21 (19.4)	1 (50.0)	0	3 (27.3)	4 (22.2)
Stage IV	29 (39.7)	14 (50.0)	0	43 (39.8)	0	1 (20.0)	0	1 (5.6)
Unknown	0	1 (3.6)	0	1 (0.9)	0	1 (20.0)	0	1 (5.6)
Mean survival ± SD (mo)	14.2 ± 13.1	15.5 ± 12.5[g]	21.0 ± 11.6	15.0 ± 12.8	17.0 ± 14.1	3.0 ± 2.2[g]	21.3 ± 10.9	16.5 ± 12.1

[a] P = 0.000001, K-*ras* mutation in 2 of 75 (2.7%) gallbladder, 5 of 33 (15.2%) bile duct, and 11 of 18 (61.1%) ampullary carcinomas.
[b] P = 0.04, K-*ras* mutation in 6 of 73 (8.2%) female patients *versus* 12 of 53 (22.6%) male patients.
[c] P = 0.008, K-*ras* mutation in 4 of 69 (5.8%) patients with gallstones *versus* 13 of 54 (24.1%) patients without gallstones.
[d] Six mucinous, 1 intestinal, and 1 clear cell adenocarcinomas; K-*ras* mutation in one ampullary mucinous adenocarcinoma.
[e] Eight adenosquamous, 2 squamous, 3 small cell, 1 carcinosarcoma, and 1 undifferentiated carcinomas; K-*ras* mutation in one ampullary small cell carcinoma.
[f] TNM, Tumor-Node-Metastasis.
[g] P = 0.03, comparison of survival in patients with and without K-*ras* mutation by product-limit method.

study and correlated the findings with the patient demographics and with the clinicopathological characteristics of the tumors.

PATIENTS AND METHODS

Patient Population. Patients with primary biliary tract cancer (ICD9, 156) newly diagnosed between 1997 and 1999 were identified through a rapid-reporting system established between the Shanghai Cancer Institute and 30 collaborating hospitals in urban Shanghai. This reporting system recruited >95% of patients with biliary tract cancers in Shanghai. A total of 126 patients with biliary tract cancers were included in this analysis. These patients were identified as part of a large, population-based, case-control study. Eligibility criteria for recruitment of patients for this study included the following: residents of urban Shanghai between 18 and 74 years of age diagnosed after April 1997 with gallbladder, extrahepatic bile duct, or ampullary carcinomas. The study was approved by the National Cancer Institute Institutional Review Board.

Tissue Specimens. Surgical pathology specimens were collected from biliary tract cancer patients who were undergoing curative resection by pancreaticoduodenectomy, bile duct resection, or biopsy of an advanced tumor. As part of the case-control study, 6 H&E-stained slides and 6 unstained slides (5 μm each) and 10 ml of peripheral blood were routinely collected from each patient from the surgical pathology and surgery departments of the participating hospitals. In addition, a structured questionnaire was used to elicit information on demographic, clinical, and epidemiological variables. The anatomical location where the tumor was taken was recorded in a diagram completed by the local pathologist at the participating hospital. The histopathological slides were reviewed by two pathologists from Shanghai and were independently reviewed by one of us (A. R.). Classification was based on the WHO classification of tumors of the biliary tract (40). Medical records were abstracted for all cancer cases. Patients' follow-up was obtained by the Shanghai Cancer Institute by contacting the patients or their relatives in the time interval from the date of diagnosis to March 2001.

DNA Preparation. Genomic DNA was extracted from carcinomas by microdissection from H&E-stained slides without coverslip obtained from paraffin embedded, formalin-fixed tissue and prepared as described in previous studies (41). The neoplastic cellularity of the areas selected for microdissection was >50%. DNA from peripheral blood was used but was unavailable from 28 patients.

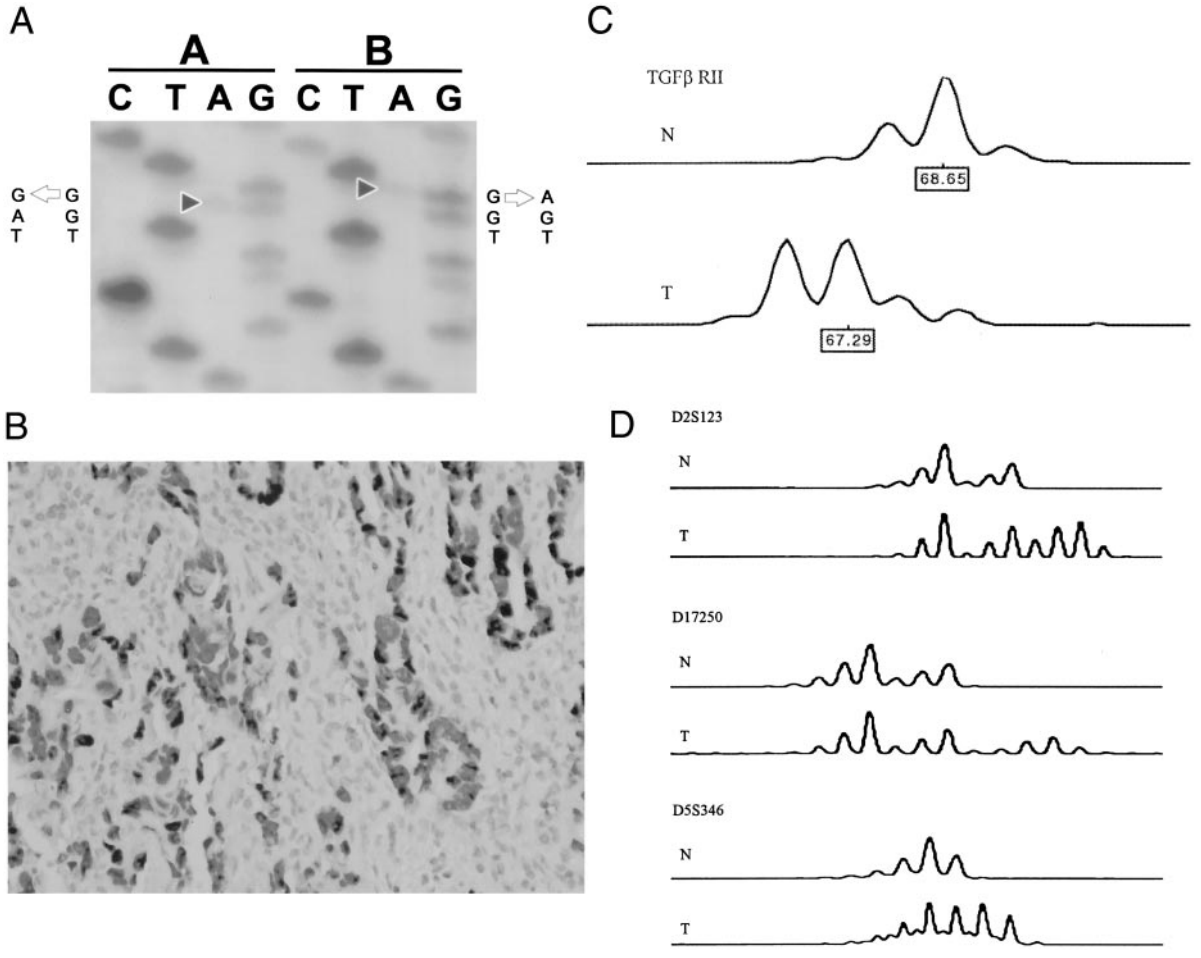

Fig. 1 Genetic alterations in biliary tract cancers. *A,* sequence of K-*ras* gene, exon 1, showing two cancers (*A* and *B*) with G to A transition at codon 12, second nucleotide (substitution of amino acid glycine with aspartic acid); and a G to A transition at codon 12, first nucleotide (substitution of amino acid glycine with serine). *B,* immunohistochemistry for p53 showing intense, nuclear staining of the neoplastic glands. *C,* PCR products from amplification of 73-bp segment of *TGF*β *type II receptor* gene (no. 2, Table 3). The PCR product from tumor has a 2-bp deletion. *N,* normal blood DNA; *T,* tumor DNA. *D,* MSI-high carcinoma with allelic shifts in three dinucleotide markers (no. 4, Table 3). The dinucleotide markers are shown *above* the electropherogram. *N,* normal blood DNA; *T,* tumor DNA.

Point Mutations of the K-*ras* Proto-oncogene. The first exon of K-*ras* was amplified, and all possible point mutations in codons 12 and 13 were tested by DNA sequencing, as described previously (42).

Immunohistochemistry for p53 Overexpression. Immunohistochemistry with mouse monoclonal antibody D07 (1:250 dilution) and standard techniques including antigen retrieval was used to determine p53 gene product overexpression, as in our previous studies (43). Overexpression of p53 was considered to be present when >50% of the nuclei of tumor cells were stained by immunohistochemistry.

Microsatellite Markers and PCR Amplification. Fluorescent dye-labeled PCR amplification was performed using the markers recommended by the National Cancer Institute workshop (26). The fluorescent dye-labeled and unlabeled primers were obtained (Life Technologies, Inc., Gaithersburg, MD, or Applied Biosystems, Foster City, CA). The 5′ oligonucleotide was end-labeled with 6-carboxyflourescein (*BAT-25*, *BAT-26*, *D17S250*, and *TGF*β*RII*), HEX (*D5S346*), or TET (*D2S123*) fluorescent dyes. PCR was performed in 15-μl reaction volumes containing 40 ng of DNA, 9 μl of ABI Prism True Allele PCR premix (Applied Biosystems, Foster City, CA), and 5 pmol of each primer. PCR was performed using a GENEAMP PCR system 9700 thermal cycler (Applied Biosystems) using the following cycling conditions: denaturation at 95°C for 12 min; 10 cycles of 94°C for 15 s, 55°C for 15 s, and 72°C for 30 s; 32 cycles of 89°C for 15 s, 55°C for 15 s, 72°C for 30 s; and a final extension at 72°C for 10 min. A 1.0-μl aliquot of each fluorescent dye-labeled PCR product was combined with 12 μl of formamide and 0.5 μl of GENESCAN 400HD [ROX] size standard (Applied Biosystems) and analyzed on an ABI 3700 Genetic Analyzer using GeneScan Analysis software (Applied Biosystems).

Alteration of the *TGF*β*RII* Gene. Alteration in the 10-bp polyadenine tract present in the third exon of the *TGF*β*RII* gene was studied by PCR amplification of a 73-bp

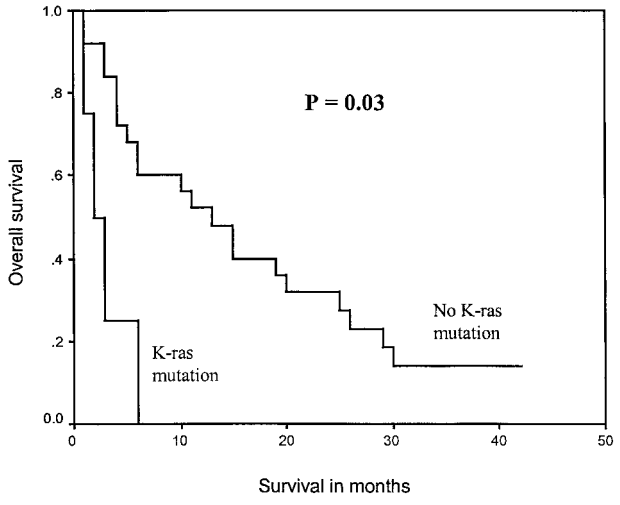

Fig. 2 Overall survival of patients with bile duct carcinomas and K-*ras* mutation status. The mean survival of patients with K-*ras* mutation in cancer was 3.0 ± 2.2 months compared with 15.5 ± 12.5 months for patients without K-*ras* mutation ($P = 0.03$, product-limit method).

region, as described previously (44). Mutation was defined as an additional peak to the right (insertion) or left (deletion) of the wild-type allelic band. To confirm that alteration of the *TGF*β*RII* gene was attributable to mutation of the polyadenine tract, DNA sequencing was performed. The 73-bp region was amplified by PCR using unlabeled primers and sequenced, as described previously (16). Each mutation was verified by sequencing in both directions.

MSI. The presence of MSI was determined from the PCR amplifications of the six microsatellite markers: two mononucleotide markers (*BAT-25* and *BAT-26*), three dinucleotide markers (*D2S123*, *D5S346*, and *D17S250*) recommended by the National Cancer Institute workshop (26), and *TGF*β*RII*. MSI-high was defined by allelic shifts in 40% or more of the evaluated dinucleotide markers or alteration of mononucleotide markers including *TGF*β*RII* if peripheral DNA was unavailable. MSI-low was defined by allelic shifts in <40% of the evaluated dinucleotide markers. MSI-low cancers were considered with microsatellite stable cancers.

Statistical Analysis. The statistical analysis for each genetic alteration was performed with clinical or pathological characteristic by χ^2 or Fisher's exact tests using genetic status as a dichotomous variable and age as a continuous variable by Student's *t* test. Overall survival time was measured from the date of surgery and compared using the product-limit method (45). These analyses also were evaluated for each tumor subsite.

RESULTS

K-*ras* Mutation. Mutation of K-*ras* was present in 18 of 126 (14.3%) biliary tract cancers (Table 1 and Fig. 1*A*). Fifteen cancers had mutations in the second nucleotide of codon 12, and 9 were attributable to a G to A transition at this nucleotide, resulting in the substitution of aspartic acid for glycine. Mutation in K-*ras* was dependent on the subsite of tumor; mutation was present in 11 of 18 (61.1%) ampullary cancers but in only 5 of 33 (15.2%) bile duct cancers and 2 of 75 (2.7%) gallbladder cancers ($P = 0.000001$; Table 1).

The K-*ras* mutation status of the cancer was also associated with gender and gallstone status. K-*ras* mutation was present in 6 of 73 (8.2%) female patients but in 12 of 53 (22.6%) male patients ($P = 0.04$). Similarly, K-*ras* mutation was present in 4 of 69 (5.8%) patients with gallstones but in 13 of 54 (24.1%) patients without gallstones ($P = 0.008$). The associations of K-*ras* mutation status with gender or gallstones were not significant when the tumor site was taken into consideration. The mean survival of patients who had bile duct carcinomas with K-*ras* mutation was 3.0 ± 2.2 months compared with 15.5 ± 12.5 months for those without mutation ($P = 0.03$; Fig. 2; Table 1). The mean survival of patients with or without K-*ras* mutation was not different for gallbladder or ampullary carcinomas.

p53 Overexpression. Normal bile ducts, gallbladder, or duodenum had no staining for p53 by immunohistochemistry, but p53 overexpression was present in 34 of 123 (27.6%) biliary tract cancers (Fig. 1*B*). p53 overexpression was present in 30 of 100 (30.0%) adenocarcinomas not otherwise specified and papillary adenocarcinomas but was absent in 8 adenocarcinomas of other subtypes ($P = 0.02$; Table 2). p53 overexpression in cancer was not associated with other patient or tumor characteristics or with prognosis.

MSI. MSI-high was present in 4 of 126 (3.2%) biliary tract cancers (Table 3), and MSI-low was present in 1 of 126 (0.8%) biliary tract cancers without HNPCC. Alteration of the *TGF*β*RII* gene was present in 3 of 126 (2.4%) cancers (Fig. 1*C*), *BAT-26* in one of 126 (0.8%), and *BAT-25* in 0 of 126 cancers. Three of 98 (3.1%) cancers had allelic shifts in the dinucleotide markers. One cancer with alteration of *TGF*β*RII* gene and *BAT-26* had shifts of all three dinucleotides markers. The other two cancers had shifts of three of three dinucleotide markers (Fig. 1*D*) and one dinucleotide marker without alterations of the *TGF*β*RII* gene or *BAT* loci. DNA from peripheral blood was unavailable from two of three cancers with alteration of the *TGF*β*RII* gene, but frameshift mutations of the polyadenine tract were confirmed by DNA sequencing in all three cancers.

MSI-high in cancer was associated with histology of cancer and age of onset (Table 3). MSI-high was present in 2 of 6 (33.3%) mucinous adenocarcinomas but in only 2 adenocarcinomas not otherwise specified among 118 (1.7%) cancers of other types ($P = 0.006$; Fig. 3). MSI-high cancers were present in 2 of 11 (18.2%) patients <50 years of age but in only 2 of 115 (1.7%) patients 50 years or older ($P = 0.04$). The age of onset of cancer was 55.2 ± 8.9 years for patients with MSI-high cancers and 65.0 ± 8.9 years for patients with microsatellite-stable cancers ($P = 0.04$). The prognosis of patients with MSI-high cancers was heterogeneous; 1 patient with MSI-high cancer was alive for 37 months, 1 patient died 13 months after surgery, and the remaining 2 patients died but the survival period was unavailable.

DISCUSSION

The molecular genetics of biliary tract carcinomas has been studied extensively, but few studies have addressed clinical and

Table 2 Prevalence of p53 overexpression compared with patient demographics and carcinoma characteristics in 123 biliary tract carcinomas

	p53 overexpression no. (%)							
	Absent				Present			
	Gallbladder (n = 49)	Bile duct (n = 26)	Ampulla (n = 14)	All sites (n = 89)	Gallbladder (n = 23)	Bile duct (n = 7)	Ampulla (n = 4)	All sites (n = 34)
Age (mean ± SD) yr	66.1 ± 8.8	62.7 ± 9.9	68.1 ± 5.8	65.5 ± 8.8	63.6 ± 9.9	63.7 ± 8.7	65.8 ± 9.2	63.9 ± 9.1
Gender								
Female	36 (73.5)	7 (26.9)	6 (42.8)	49 (55.1)	19 (82.6)	4 (57.1)	0	23 (67.6)
Male	13 (26.5)	19 (73.1)	8 (57.1)	40 (44.9)	4 (17.4)	3 (42.9)	4 (100.0)	11 (32.4)
Cholelithiasis								
Present	35 (71.4)	9 (34.6)	5 (35.7)	49 (55.1)	14 (60.9)	3 (42.9)	0	17 (50.0)
Absent	12 (24.5)	16 (61.5)	9 (64.3)	37 (41.6)	9 (39.1)	4 (57.1)	4 (100.0)	17 (50.0)
Unknown	2 (4.1)	1 (3.8)	0	3 (3.4)	0	0	0	0
Histological type, Adenocarcinoma								
Not otherwise specified	29 (59.2)	21 (80.8)	11 (78.6)	61 (68.5)[a]	17 (73.9)	5 (71.4)	4 (100.0)	26 (76.5)[a]
Papillary	7 (14.3)	1 (3.8)	1 (7.1)	9 (10.1)[a]	4 (17.4)	0	0	4 (11.8)[a]
Other adenocarcinomas	5 (10.2)[b]	2 (7.7)[b]	1 (7.1)	8 (9.0)[a,b]	0	0	0	0[a]
Other histological types	8 (16.3)[c]	2 (7.7)[c]	1 (7.1)[c]	11 (12.4)[c]	2 (8.7)[c]	2 (28.6)[c]	0	4 (11.8)[c]
Mean survival ± SD (mo)[d]	13.6 ± 12.5	14.3 ± 12.1	21.0 ± 11.7	15.8 ± 12.9	12.6 ± 11.5	12.1 ± 14.1	21.7 ± 8.3	13.1 ± 11.4

[a] $P = 0.02$, p53 overexpression in 0 of 8 adenocarcinomas of other histological type *versus* 30 of 100 (30%) in adenocarcinomas not otherwise specified and papillary adenocarcinomas.
[b] Six mucinous, 1 intestinal, and 1 clear cell adenocarcinomas.
[c] Seven adenosquamous, 2 squamous, 4 small cell, 1 carcinosarcoma, and 1 undifferentiated carcinomas; p53 overexpression in 1 small cell carcinoma and 1 carcinonosarcoma of the gallbladder, and 2 adenosquamous carcinomas of the bile duct.
[d] Product-limit method.

Table 3 Clinicopathological features of carcinomas with MSI-high

No.	Age	Sex	Tumor site	Histological type of cancer	Shift of mononucleotide markers	Shift of dinucleotides markers	K-*ras* mutation	HNPCC	Survival status
1	69 yr	Male	Gallbladder	Mucinous	Present[a]	NP[b]	Absent	Absent	Dead, 13 mo
2	65 yr	Female	Bile duct	Mucinous	Present[a,c]	Present	Absent	Absent	Dead, unknown
3	38 yr	Male	Bile duct	NOS[b]	Present[a]	NP	Absent	Absent	Dead, unknown
4	49 yr	Male	Gallbladder	NOS	Absent	Present	Absent	Absent[d]	Alive, 37 mo

[a] *TGF*β*IIR*.
[b] NOS, not otherwise specified; NP, not performed.
[c] *BAT-26*.
[d] Patient's father had a lung carcinoma.

pathological associations in prospectively defined patient populations. We, therefore, evaluated K-*ras* point mutations, p53 overexpression, and MSI, including alterations of the polyadenine tract of the *TGF*β*RII* gene, in 126 biliary tract cancers from a population-based study.

K-*ras* mutation was present in 14.3% of the biliary tract cancers in our study. The frequency of K-*ras* mutation was dependent on the subsite of cancer and was present in 61.1% of ampullary cancers but only 15.2% of bile duct and 2.7% of gallbladder cancers. The reported prevalence of K-*ras* mutation is 19–41% for ampullary carcinomas (5, 6, 9–11), 0–39% for bile duct carcinomas (3, 4, 7, 9, 12, 13), and 0–100% for gallbladder carcinomas (3, 7–9). These variations in K-*ras* mutation rates in biliary tract carcinomas may be attributable to the use of different assay techniques in these studies or to racial and geographical variations in the study populations. Similar to our study, the reported prevalence of K-*ras* mutation was lower when sequencing was used for detecting mutation (3, 5, 6, 8, 13), compared to when either restriction fragment length polymorphism, PCR mismatch amplification, or denaturing gradient gel electrophoresis was used to detect mutation (4, 7, 9, 10, 12). Racial or geographical variations in K-*ras* rates were reported in cholangiocarcinomas from Thailand and Japan (46). Similar to our study, a lower prevalence of K-*ras* mutation in gallbladder carcinomas has been reported (3, 8, 14); and the reported prevalence of K-*ras* mutation was lower in the gallbladder carcinomas compared with the bile duct carcinomas (3, 7, 9).

Similarly, lesions that are precursors of invasive carcinomas of the biliary tract show differences in K-*ras* mutations in the different subsites. Dysplasia and carcinoma *in situ* of the

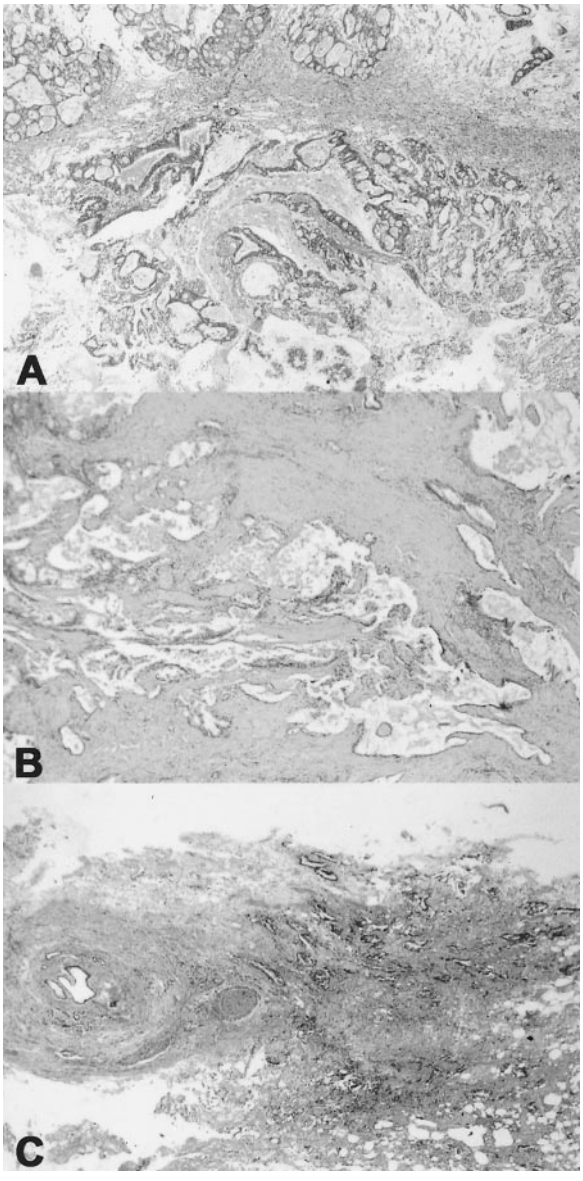

Fig. 3 Histology of carcinomas with alterations of *TGF* β *type II receptor* gene. *A* and *B*, mucinous carcinomas of gallbladder and hepatic ducts, respectively (nos. 1 and 2, Table 3). *C*, an adenocarcinoma, not otherwise specified, of pancreatic bile duct (no. 3, Table 3).

gallbladder, precursors of invasive gallbladder carcinomas, lack K-*ras* mutations (47), but gallbladder adenomas, which are not considered precursors of invasive gallbladder carcinoma, do have K-*ras* mutations (48). In contrast, K-*ras* mutations are commonly present in ampullary adenomas and in carcinomas with an adenomatous component but are uncommon in ampullary carcinomas without an adenomatous component (6, 10, 11). These data suggest that two parallel pathways of carcinogenesis are operative in the biliary tract; the majority of gallbladder and bile duct carcinomas develop from a K-*ras*-independent pathway, whereas carcinomas of the ampulla develop from a K-*ras*-dependent pathway with an adenoma-carcinoma sequence. K-*ras* mutations are frequent in gallbladder and bile duct carcinomas arising in patients with an anomalous junction of the pancreaticobiliary duct (49, 50), suggesting that a K-*ras*-dependent pathway also exists in the pathogenesis of gallbladder and bile duct tumors.

In our study, the lack of K-*ras* mutations in female patients and in patients with gallstones was attributable to a strong association between gallbladder cancer and gender and gallstone status. These associations were not present when K-*ras* mutation status was considered for each cancer subsite. In our study, K-*ras* mutation status was a prognostic factor for patients with bile duct carcinomas but not for patients with gallbladder or ampullary carcinomas. However, this finding was based on a limited sample site. Similarly, previous studies have reported poor survival of patients with K-*ras* mutation in biliary tract carcinomas (9) and extrahepatic bile duct carcinomas (12). In contrast, another study reported a lack of prognostic implications for the K-*ras* mutation in extrahepatic bile duct carcinomas (51).

p53 overexpression was present in 27.6% of biliary tract cancers. The reported frequency of p53 overexpression in the biliary tract ranges between 38 and 66% (19–22). p53 overexpression was present in adenocarcinomas not otherwise specified and of papillary type but not in other histological types of adenocarcinomas in our study. In a previous report, p53 overexpression and mutation were more common in "polypoid" macroscopic types of gallbladder cancers (17). p53 overexpression was not associated with other clinical or tumor characteristics or with prognosis. The lack of prognostic significance of p53 overexpression has been reported previously (52).

In our study, MSI-high was present in 3.2% of biliary tract cancers, including alteration of the polyadenine tract of the *TGF*β*RII* gene in 3 cancers. In contrast, previous studies of biliary tract cancers have reported MSI of dinucleotide repeats (37–39), but alterations of the *TGF*β*RII* gene have not been reported (37, 38). Alterations of the *TGF*β*RII* gene are frequent in colorectal and gastric MSI-high cancers but is infrequent in endometrial cancer (36).

In our study, MSI-high was associated with the mucinous histological subtype of biliary tract cancers and long survival in one of the two patients with follow-up. Similarly, MSI-high colorectal and pancreatic cancers are associated with a distinctive phenotype, genotype, and better survival (26, 53–57). HNPCC kindreds have increased incidence of biliary tract cancer (26), but there was no family history of HNPCC cancers in patients with MSI-high cancers in our study.

Geographical and/or racial differences have been reported in the frequencies of the genetic alterations of biliary tract cancers studied in the present study. However, these frequencies in our study are not markedly different from that reported in the literature and do not explain the rising incidence of biliary tract cancer in Shanghai, China.

In conclusion, genetic alterations in biliary tract cancer vary by subsite and histological type of tumor and by age of onset of the tumor. The frequency of the K-*ras* mutation is dependent on the subsite of the biliary tract cancer and associated with prognosis of bile duct cancers; p53 overexpression and MSI are associated with the histological type of tumor; and MSI is associated with early age of onset without HNPCC.

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